Ovarian Responses to Intramuscular or Intravenous Administration of Prostaglandin F{alpha}2 in Control and FSH-Treated Beef Heifers

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OVARIAN RESPONSES TO INTRAMUSCULAR OR INTRAVENOUS ADMINISTRATION OF PROSTAGLANDIN $F_{2\alpha}$ IN CONTROL AND FSH-TREATED BEEF HEIFERS

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ABSTRACT

Sixty Simmental crossbred heifers 18 to 20 mo of age were detected in estrus and assigned at random to a 2 × 2 factorial design study with 30 controls, and 30 given FSH. Half of each group was given prostaglandin $F_{2\alpha}$ (PGF) i.v. and the rest i.m. Injections of follicle-stimulating hormone were started on d 7 to 14 of an estrous cycle and continued for 5 d or until ovariectomy; PGF was administered either i.v. or i.m. at 48 (25 mg) and 60 (15 mg) h after the initial FSH injection. Control females received a similar PGF treatment on a day between d 9 and 15 of the estrous cycle. Blood samples were collected from all animals immediately before PGF administration and every 12 h thereafter until ovariectomy. Within each of the four experimental subgroups, ovariectomies were performed at either 24, 48 or 72 h (five/time group) after initial PGF injection. Ovarian and corpus luteum (CL) weights were recorded as well as number and size of follicles and number of ovulations. Regression of the CL was slower ($P < .05$) after administration of PGF i.v. than i.m. (CL weight was 2.6 vs 3.3 ± .2 g for i.m. and i.v. groups, respectively). Exogenous FSH increased estradiol-17β (E2) concentrations, and FSH-treated heifers had more ($P < .05$) early ovulations than control heifers did. Ovulations in FSH-treated heifers had begun to occur by 24 h after i.v. and i.m. PGF injection. This investigation showed that route of PGF administration can alter CL regression, because females receiving PGF i.v. had heavier CL weights and a smaller percentage reduction in concentration of P4 at 24 h after PGF injection.

(Key Words: Prostaglandin $F_{2\alpha}$, Superovulation, Corpus Luteum, Heifers, Injection.)

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Introduction

Prostaglandin $F_{2\alpha}$ (PGF) or its analogues have been used to synchronize estrus in cattle since the early 1970s (Tervit et al., 1973; Louis et al., 1974) and have been used extensively for embryo transfer to synchronize estrus in donor females. Changes in embryo quality in nonsuperovulated and superovulated bovine females have been associated with asynchronous endocrine events and asynchronous ovulation (Jensen et al., 1982; Maurer and Echternkamp, 1982; Donaldson, 1985). Route of administration and dosage of prostaglandins produced differences in timing of progesterone disappearance and estrogen and LH rises during the preovulation sequence (Stellflug et al., 1973; Chenault et al., 1976). Maurer and Echternkamp (1982) reported that the percentage of viable embryos appeared to be greater in superovulated females receiving PGF intravenously compared with females receiving PGF intramuscularly. Donaldson

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(1983) showed that multiple injections of PGF increased the number of superovulated females expressing estrus and also increased the number of viable embryos. Therefore, our experiment was conducted to determine whether the route of administration of PGF influenced synchrony of endocrine changes and synchrony of ovulation in FSH-treated heifers.

Materials and Methods

Sixty crossbred heifers (Simmental sired) 18 to 20 mo of age were detected in estrus and assigned randomly to a 2 × 2 factorially designed study that included a control group and a group treated with FSH; each group was given PGF either i.v. or i.m. Follicle-stimulating hormone[4] was administered i.m. starting between d 7 and 14 of the estrous cycle and was continued for 5 d or until ovariectomy. The dosage administered was 5, 4, 3, 2 and 2 mg twice daily (total 32 mg). Fifteen heifers from each group received PGF[5] via i.v. (jugular vein) or i.m. route. Prostaglandin $F_{2\alpha}$ was administered at 48 (25 mg) and at 60 (15 mg) h after the initial FSH injection. Control heifers received PGF (25 and 15 mg at a 12-h interval) on a day between d 9 and 15 of the estrous cycle. Animals were observed for estrous behavior for 1 h daily. A blood sample was collected from a jugular vein of each heifer immediately before the first PGF injection and every 12 h thereafter until ovariectomy. Within each of the four experimental subgroups, ovariectomies were performed at 24, 48 or 72 h (five heifers/time group) after the initial PGF injection. Ovariectomy was performed by making a 5- to 8-cm incision in the anterior vagina after each heifer received an epidural injection of 5 ml of the anaesthetic procaine hydrochloride. Ovaries were removed by cutting and crushing the tissue at the ovarian hilus with a serrated, curved spay scissors. Ovaries were collected to determine whether ovulation occurred synchronously or asynchronously. Each ovary was weighed and the regressing corpus luteum (CL) was dissected and weighed; ovulations and number of follicles of various size (1 to 3, 4 to 7 and 8 mm or greater in diameter) were recorded.

Progesterone (P4) and estradiol-17β (E2) were assayed in each serum sample. Progesterone and E2 concentrations were determined using RIA procedures described by Maurer and Echternkamp (1982, 1985). A P4 antibody against progesterone-11α bovine serum albumin[6] was used to assay P4; the E2 content was assayed by a RIA procedure described by Kesler et al. (1977) using an antisera against E2 provided by Dr. Norman Mason[7].

Ovarian data were analyzed using a 2 × 2 × 3 fixed model least squares analysis (Harvey, 1975); P4 and E2 data were analyzed using a split plot over time mixed model least squares analysis with heifers within treatment as the random variate. Heifers-within-treatment × route of administration was the error term used to test for treatment effects, route of administration effects, and treatment × route of administration interaction. All three-way interactions were included in the residual sums of squares. Progesterone data also were analyzed using time as a covariate to determine whether the slopes of the four subgroups differed.

Results

Day of the estrous cycle when PGF initially was administered averaged 12.5 ± .4 and 11.9 ± .4 ($\bar{x}$ ± SE) for FSH-treated and control groups, respectively, and 12.2 ± .4 and 12.2 ± .4 for i.m. and i.v. administration, respectively. No heifers were detected in estrus at 24 or 48 h after PGF injection. At 72 h, six FSH-treated (three i.v. and three i.m. PGF) and three control (two i.v. and one i.m. PGF) heifers were detected in estrus. No differences in detection of estrus were found ($P > .10$) between FSH-treated and control females or between heifers administered PGF i.m. or i.v.

Ovarian data collected from FSH-treated and control heifers given PGF either i.v. or i.m. are given in Table 1. Total ovarian weight was heavier ($P < .001$) in FSH- treated than in control heifers (34.2 ± 3.2 vs 15.2 ± 3.2 g, respectively). Corpora lutea weight did not differ between FSH-treated and control groups but were heavier ($P < .05$) with i.v. than i.m. PGF (3.3 ± .2 vs 2.6 ± .2 g). Corpora lutea weight differed with time ($P < .001$), but interactions were not significant. Heifers treated with FSH had more ($P < .05$; Table 2)

[4]FSH-P®, Burns Biotec (Schering Corp. U.S.A.), Omaha, NE.
[5]Lutalyse®, The Upjohn Company, Kalamazoo, MI.
[6]Miles-Yeda Ltd., Israel.
[7]Eli Lilly Co., Indianapolis, IN.

TABLE 1. OVARIAN AND CORPORA LUTEA WEIGHTS, NUMBER AND SIZE OF FOLLICLES BY TREATMENT, TIME AND ROUTE OF PGF ADMINISTRATION[a]

	PGF administration						
	i.m.			i.v.			
	Hours from initial PGF to ovariectomy						
Item	24	48	72	24	48	72	SE[b]
Control							
No. of females	5	5	5	5	5	5	
Ovarian wt, g	14.9	19.0	13.8	18.6	12.7	11.9	8.1
Corpus luteum wt, g	3.71	1.89	2.08	5.82	2.72	1.82	.62
No. follicles > 8 mm	2.2	2.8	3.6	2.4	3.6	2.6	4.5
No. follicles 4-7 mm	4.0	5.0	5.0	6.0	5.4	3.0	3.9
No. follicles 1-3 mm	24.0	40.4	21.2	24.2	12.4	10.0	6.2
Total follicles	30.2	48.2	29.8	32.6	21.4	15.6	9.1
FSH							
No. of females	5	5	5	5	5	5	
Ovarian wt, g	26.0	39.7	25.2	32.9	41.8	39.6	8.1
Corpus luteum wt, g	3.79	2.65	1.45	5.01	2.86	1.74	.62
No. follicles >8 mm	20.4	25.6	18.6	14.4	21.4	30.0	4.5
No. follicles 4-7 mm	11.0	5.6	6.4	18.2	8.4	8.0	3.9
No. follicles 1-3 mm	2.0	.6	1.0	1.4	3.8	1.4	6.2
Total follicles	33.4	31.8	26.0	34.0	33.6	39.4	9.1

[a]Least square means.

[b]Least square standard errors.

ovulations than the control heifers when averaged for the three ovariectomy groups (4.0 vs .1 ovulations, respectively). More FSH-treated heifers (24/30) had early ovulations than control heifers (4/30; Table 2). Route of PGF administration did not influence ($P > .10$) number of ovulations. Although average number of ovulations increased with time (Table 2), statistically there was no difference ($P > .10$; .8, 1.4 and 4.2 ± 1.3; $\bar{x}$ ± SE among 24, 48 and 72 h ovariectomies, respectively).

The interaction ($P < .10$) of treatment and PGF administration in total number of follicles suggested that PGF given i.v. permitted more follicles to grow in FSH-treated females but not in the control group, which received PGF i.v. (Table 1). Total number of follicles did not differ ($P > .10$) between FSH or control groups; however, treatments differed within different follicular sizes ($P < .05$). Ovaries from the FSH-treated group had more 8-mm or greater ($P < .001$) and 4- to 7-mm ($P < .05$)

TABLE 2. NUMBER OF HEIFERS WITH OVULATIONS AND AVERAGE NUMBER OF OVULATIONS BY TREATMENT, TIME AND ROUTE OF PGF ADMINISTRATION

	PGF administration						
	i.m.			i.v.			
	Hours from initial PGF to ovariectomy						
Item	24	48	72	24	48	72	SE
Control							
No. of females	5	5	5	5	5	5	
No. of females with ovulations	1	0	1	0	0	2	
No. of ovulations/female	.2	0	.2	0	0	.4	.06
FSH							
No. of females	5	5	5	5	5	5	
No. of females with ovulations	3	4	4	4	4	5	
No. of ovulations/female	1.4	3.2	4.0	1.4	2.2	12.0	1.54

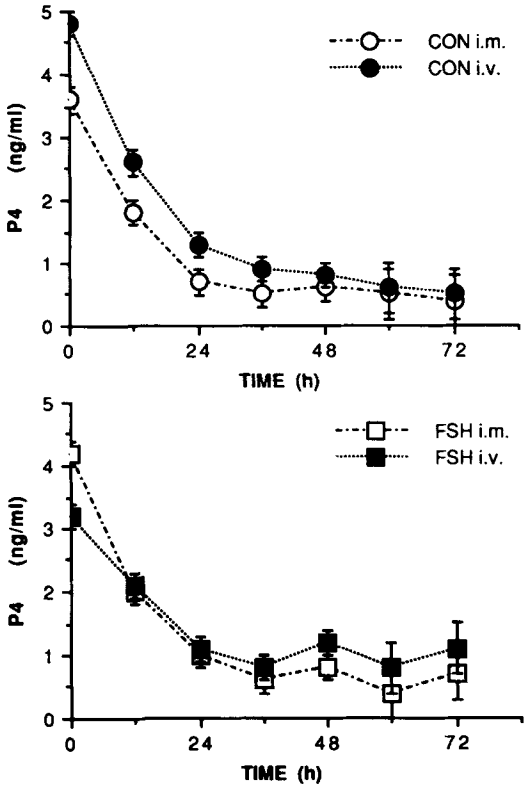

Figure 1. Progesterone (P4) (ng/ml) by time in FSH-treated (FSH) and control (CON) heifers given PGF either intramuscularly (i.m.) or intravenously (i.v.). Vertical bars are SE.

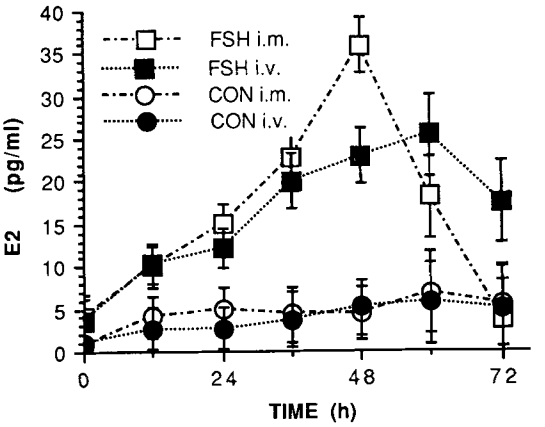

Figure 2. Estradiol-17β (E2) (pg/ml) by time in FSH-treated (FSH) and control (CON) heifers given PGF either intramuscularly (i.m.) or intravenously (i.v.). Vertical bars are SE.

but fewer 1- to 3-mm ($P < .001$) follicles than ovaries from control heifers.

The P4 concentrations are shown in Figure 1. Concentrations of P4 decreased ($P < .01$) over time. Progesterone did not differ ($P > .10$) by treatment (1.4 vs 1.4 ng/ml over time for FSH-treated and control heifers, respectively) or by i.m. or i.v. administration of PGF (1.3 vs 1.6 ± .1 ng/ml over time, respectively), but the interaction of treatment × PGF administration approached significance ($P < .10$). Heifers treated with FSH that received PGF by the i.v. route had lower concentrations of P4 initially and greater concentrations at 72 h, whereas in control heifers those females that received PGF i.v. had greater P4 values initially and concentrations similar to those of females receiving PGF i.m. at 72 h. Using time as a covariate, a significant interaction of time × treatment × route of administration indicated that the slopes of the P4 concentration over time were different. Intramuscular administration of PGF caused a steeper decline in P4 concentration than did i.v. administration of PGF.

Estradiol-17β (Figure 2) was affected by time ($P < .01$), treatment ($P < .001$; 15.7 vs 4.0 ± 1.0 pg/ml over time for FSH and control heifers, respectively) and the interaction ($P < .001$) of treatment × time. Heifers treated with FSH had higher E2 concentrations sooner than did the heifers in the control group.

Discussion

Route of administration of PGF caused the corpus luteum to regress at different rates. Corpus luteum regression was slower in females that received PGF i.v. This most likely occurred because most of the i.v. injected PGF probably would be metabolized during the first few passages through the lungs, resulting in a shorter peripheral exposure, whereas i.m. injected PGF would be released slowly from the injection site, resulting in a longer exposure. The lungs are a main organ in PGF metabolism (Oesterling et al., 1972; Anderson and Eling, 1976), and PGF injected into the jugular vein would be metabolized in the lungs quickly. Chenault et al. (1976) reported that E2 increased faster in females that received intrauterine administration of 10 mg PGF compared with 30 mg PGF i.m., indicating that uterine administration of PGF was more

effective. Animals receiving PGF via intrauterine administration also tended to express estrus earlier, had their LH surge sooner and ovulated before females receiving PGF i.m. Similarly, Stellflug et al. (1973) found shorter intervals to estrus, to peak LH and to ovulation in females receiving 60 mg PGF compared with a 30-mg dose, indicating that dosage of PGF can change endocrine and physiological events. Donaldson (1983) showed an increase in estrous response using multiple doses of PGF in superovulated cows. In the present study, there were no differences in the number of heifers that exhibited estrous behavior between FSH-treated or control heifers or between females receiving PGF by i.v. or i.m. routes.

Exogenous FSH increased the E2 concentrations in FSH-treated heifers compared with control heifers. The increased E2 in the FSH group results from increased numbers of medium and large follicles (Wise et al., 1986). Concentrations of P4 declined more rapidly after i.m. PGF administration than after i.v. PGF administration. Corpora lutea regression was also quicker in the females receiving PGF i.m. than in females receiving PGF i.v. The slower regression of the corpus luteum in females receiving PGF i.v. may allow the oocytes to mature similarly to those during natural estrus, thus producing more viable embryos in superovulated females. Maurer and Echternkamp (1982) reported that superovulated heifers given PGF i.v. averaged two or more viable embryos than females receiving PGF i.m. Increased concentrations of P4 before or at estrus in superovulated females affected embryo quality (Jensen et al., 1982; Greve et al., 1984b; Callesen et al., 1987). The increased P4 concentrations were associated with hormonal asynchrony (Callesen et al., 1987).

Asynchronous ovulation in superovulated cattle was reported by Callesen et al. (1987). They also reported a higher incidence of asynchronous ovulations in heifers (21%) compared with cows (3%). All heifers and cows with asynchronous ovulations had asynchronous endocrine profiles, which was assumed to be the cause of abnormal oocyte production. Callesen et al. (1987) attributed the asynchronous ovulations to the gonadotropin preparation used and to the developmental competence of ovarian follicles present at the initial gonadotropin injection. However, in our study, the increased number of asynchronous ovulations found in the FSH-treated females could have resulted from LH contamination of exogenous FSH or larger endogenous E2 concentrations, or the PGF injection could have released sufficient endogenous LH to ovulate some follicles. Estradiol and LH increase 1 to 9 h after PGF injection (Hafs et al., 1974; Chenault et al., 1976). These asynchronous ovulations could increase P4 concentrations in FSH-treated females and thus produce asynchronous endocrine profiles. Maxwell et al. (1978) showed that ovulation was 66% complete by 96 h after the PGF injection in PMSG-stimulated cows, whereas Yadav et al. (1986), using FSH and cloprostenol to superovulate beef heifers, showed that ovulation started at 64.5 h after the cloprostenol injection and was completed 12 h later. Increased concentration of P4 affected the interval of time from estrus to the LH peak and the LH peak height in superovulated females (Jensen et al., 1982; Greve et al., 1984a; Callesen et al., 1987). Increasing the interval in time from estrus to the LH surge decreased the quality of the embryos in nonsuperovulated heifers (Maurer and Echternkamp, 1982). Donaldson (1985) reported that LH peak height and duration in FSH-treated females were similar to those in control females. Donaldson (1985) also reported that within the FSH group a larger variation occurred in the interval of time from estrus to LH peak. Maurer and Echternkamp (1982) reported a large variation in the interval of time from estrus to LH peak in nonsuperovulated females.

The variability in superovulatory response may be inherent in the superovulation procedure used. Perhaps the use of highly purified FSH (low LH contamination) along with smaller doses and multiple injections of PGF may produce a more synchronous endocrine profile and thereby reduce the incidence of asynchronous ovulations. Only one control female ovulated within 48 h of the PGF injection, compared with 15 of 20 FSH-treated heifers, indicating that FSH-treated heifers tended to ovulate more asynchronously. Prostaglandin $F_{2\alpha}$ administered i.v. decreased the rate of CL regression and the associated hormonal changes, but asynchronous ovulations still occurred in FSH-treated females within 24 and 48 h after the PGF injection. Controlling the asynchrony in FSH-treated females possibly would increase embryo quality.

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